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Note

Improved high-speed counter-current chromatograph with three multilayer coils connected in series

IV. Evaluation of preparative capability with large multilayer coils

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As previously reported [1–3], an improved design of the centrifuge for high-speed counter-current chromatography (CCC) has greatly increased its partition efficiency in separations where the sample size ranges from micrograms to several hundred milligrams. In order to further extend the preparative capability of the apparatus, we have constructed a new centrifuge which can hold much longer multilayer coils for the separation of multigram quantities of samples. The performance of the apparatus has been successfully demonstrated in the separation of dinitrophenyl (DNP) amino acids using a two-phase solvent system composed of chloroform–acetic acid–0.1 *M* hydrochloric acid at a volume ratio of 2:2:1. The partition efficiencies obtained from the present apparatus are compared with those from the semianalytical and semipreparative columns previously reported.

EXPERIMENTAL

Apparatus

The design of the apparatus is almost identical to that of the original model [1] except that the revolutional radius is increased from 7.5 cm to 10 cm and the width of the column holder spool from 5 cm to 11 cm. Fig. 1 shows the photograph of the present high-speed CCC centrifuge equipped with a set of three large mutilayer coils around the rotary frame. Each multilayer coil consists of about 100 m of 2.6 mm I.D. PTFE (polytetrafluoroethylene) tubing (Zeus, Raritan, NJ, U.S.A.) forming seven layers of coil between a pair of flanges spaced 11 cm apart. Three columns are serially

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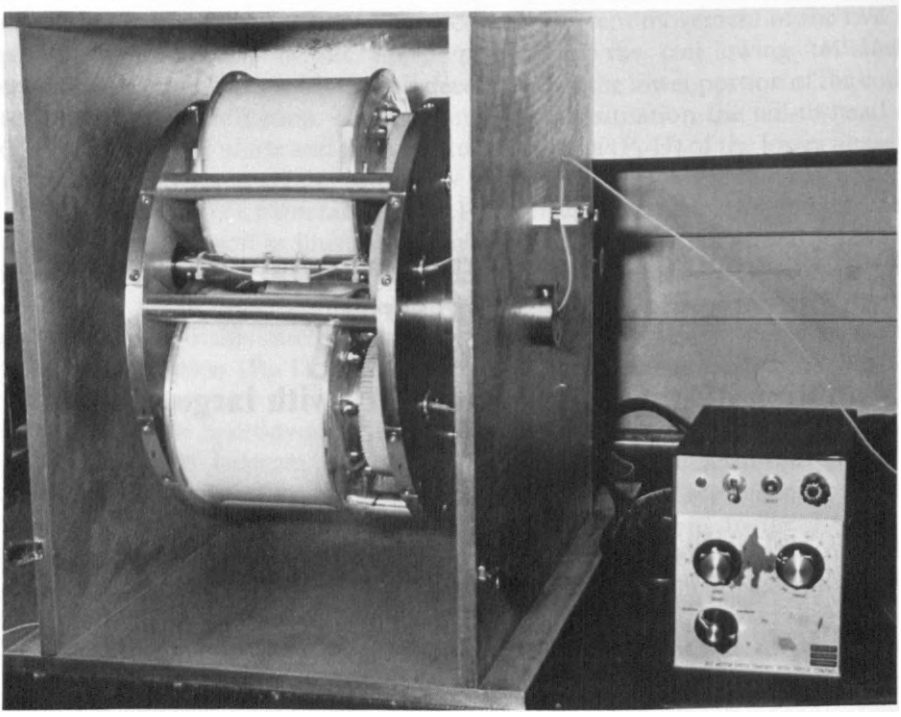

Fig. 1. Photograph of the apparatus.

connected with flow tubes to make up a total capacity of 1.6 l. The apparatus can be operated up to 1000 rpm with a speed controller (Bodine, Chicago, IL, U.S.A.).

Reagents

In the present study employed were chloroform and methanol of glass-distilled chromatographic grade (Burdick & Jackson Labs., Muskegon, MI, U.S.A.), reagent-grade glacial acetic acid (Mallinckrodt, Paris, KY, U.S.A.), 1 *M* hydrochloric acid (Sigma, St. Louis, MO, U.S.A.) and DNP-amino acid samples (Sigma) including N-2,4-DNP-D,L-glutamic acid (DNP-glu), N,N-di(2,4-DNP)-L-cystine (diDNP-$(cys)_2$), N-2,4-DNP-L-alanine (DNP-ala) and N-2,4-DNP-L-valine (DNP-val).

Preparation of two-phase solvent system and sample solution

The two-phase solvent system used in the present study was composed of chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1, v/v/v). The solvent mixture was thoroughly equilibrated in a separatory funnel by repeated shaking and degassing, and the two phases were separated shortly before being applied to the column.

The sample solution was prepared by dissolving 1 g each of DNP-val and DNP-ala, 0.2 g of diDNP-$(cys)_2$ and 2 g of DNP-glu (total weight 4.2 g) in 80 ml of the stationary upper aqueous phase.

Separation procedure

The DNP-amino acid separation was performed in the following manner: The entire column was first completely filled with the upper aqueous stationary phase; this was followed by sample injection through the sample port. The column was then rotated at the optimum speed of 740 rpm while the lower non-aqueous mobile phase was pumped into the column at a flow-rate of 7 ml/min in a head-to-tail elution mode. Here, the head-tail relationship of the rotating coil refers to an Archimedean screw force which drives all objects in the coil competitively toward the head of the coil. Effluent from the outlet of the column was monitored by absorbance at 275 nm with a UV monitor (Uvicord S; LKB, Uppsala, Sweden) and then fractionated with a fraction collector (Ultrorac, LKB). After all peaks were eluted, the apparatus was stopped and the column inlet was connected to a pressured nitrogen line (80 p.s.i.) to collect the column contents into a graduated cylinder. The volume of the stationary phase retained in the column was determined to compute the percent retention relative to the total column capacity. The column was slowly rotated in the tail-to-head elution mode to accelerate the collection of the column contents. Finally, the column was washed with about 200 ml of methanol and then dried with nitrogen.

Analyses of CCC fractions

A 20-μl aliquot of each fraction was mixed with 3 ml of methanol and the absorbance wss determined with a Zeiss PM 6 spectrophotometer at 430 nm.

RESULTS AND DISCUSSION

The performance of the present preparative high-speed CCC centrifuge was evaluated by separation of a standard set of DNP-amino acid samples. Fig. 2 shows a typical chromatogram obtained from 4 g of a mixture of DNP-amino acids using a two-phase solvent system composed of chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1, v/v/v). The separation was performed at a flow-rate of 7 ml/min using the lower non-aqueous phase as the mobile phase at a revolution speed of 740 rpm. The solvent front emerged in 75 min followed by substantial amounts of carryover of the stationary phase. Retention of the stationary phase measured 46% and the maximum pressure at the outlet of the pump was 70 p.s.i. All four components were well resolved and eluted within 8 h. The skewed peak of DNP-glu was apparently caused by a non-linear isotherm due to the high solute concentration in the sample solution.

The partition efficiencies of the separation can be computed from the chromatographic chart according to the conventional gas chromatographic formula

$$N = (4R/W)^2 \quad (1)$$

where N denotes the partition efficiency expressed in terms of theoretical plate number (TP); R the retention time or volume of the peak maximum; and W the peak width expressed in the same unit as R. The results give high partition efficiencies ranging from 1800 TP for the first peak to 1000 TP for the fourth peak. However, the absolute TP alone cannot be directly used for evaluating the performance of the apparatus since other parameters such as a length of the column, separation time, number of helical turns, etc. should also be considered. More useful expressions of the partition

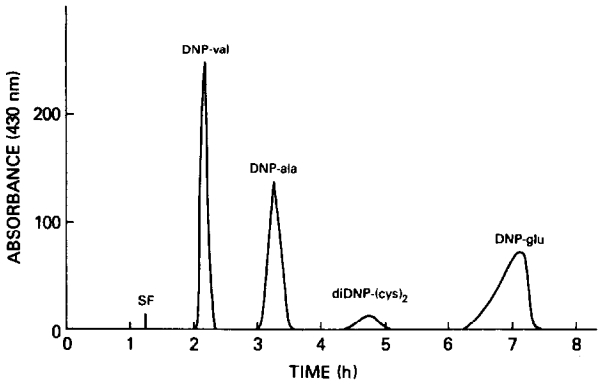

Fig. 2. Chromatogram of DNP-amino acids obtained with the present apparatus. SF = Solvent front. The experimental conditions were as follows: high-speed CCC coil planet centrifuge with 10 cm revolution radius; column, 3 multilayer coils, 2.6 mm I.D. and 1.6 l capacity with $\beta = 0.5$–0.8; β is a ratio of the rotation radius (distance from the holder axis to the coil) to the revolution radius (distance from the holder axis to the central axis of the centrifuge); solvent system, chloroform–acetic acid–0.1 *M* hydrochloric acid (2:2:1, v/v/v); mobile phase, lower non-aqueous phase; flow-rate, 7 ml/min; sample size, 4.2 g; revolution, 740 rpm; retention, 46%; pressure, 70 p.s.i.

efficiency, which incorporate these parameters, include s/TP (time required for one TP), cm/TP (a length of the column required for one TP) and TP/turn (the average TP value obtained from one helical turn of the column). Among these, s/TP is computed from the chromatogram by dividing the retention time (s) of the solvent front with the TP value of each peak.

Table I lists various partition efficiency values obtained from the present preparative column (2.6 mm I.D.) together with those similarly obtained from the semi-preparative (1.6 mm I.D.) and semi-analytical (1.07 mm I.D.) columns as previously reported in part I [1] and part III [3]. In the present preparative column, one TP is produced by a 20.5 cm length of tubing in every 3.5 s, yielding an average of 2.1 TP in each helical turn. As shown in the table, these figures are somewhat lower than those obtained from the smaller I.D. columns. The ratios in partition efficiencies between the preparative and smaller columns range from 1/2 to 1/6 in s/TP and 1/1.2 to 1/1.5 in cm/TP. The value for TP/turn for the preparative column is slightly improved over that in the semi-preparative column, apparently due to the use of a larger diameter

TABLE I

PERFORMANCE OF THREE DIFFERENT MULTILAYER COILS IN DNP-AMINO ACID SEPARATION

Multilayer coils (set of three)					Sample size	Partition efficiencies			
Type	I.D. (mm)	Length (m)	Capacity (ml)	No. of turns	(mg)	TP	s/TP	cm/TP	TP/turn
Preparative	2.6	300	1600	670	4200	1400	3.5	20.5	2.1
Semi-preparative	1.6	200	400	600	250	1125	1.7	17.8	1.9
Semi-analytical	1.07	300	270	1200	10	4000	0.6	13.3	3.3

holder spool in the preparative column which increased the length of tube per turn. However, the loss of partition efficiency in the present preparative column over the smaller I.D. columns discussed above is quite acceptable if one considers an increase in sample size from 10 mg and 250 mg to 4.2 g in the preparative column which amounts over 16 to 400 times scale up of the sample loading capacity.

CONCLUSION

The overall experimental results clearly demonstrate an excellent preparative capability of the above design. A compact bench-top model (1.5 ft. × 1.5 ft. × 1.0 ft.) of the apparatus performs multigram separation of samples at a high partition efficiency of over 1000 TP in 8 h. The present method should be useful in preparative separations of various natural and synthetic products on a laboratory scale.

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